

WHAT IS CLAIMED IS:

1. An apparatus comprising:  
a sample receptacle;  
an excitation source providing an excitation emission directed towards the sample receptacle;  
a first detector configured to detect a first sample emission;  
a second detector configured to detect a second sample emission; and  
a processor configured to calculate a ratio of the detected first sample emission to the detected second sample emission.
2. The apparatus of Claim 1, further comprising a first filter interposed between the sample receptacle and the first detector and configured to substantially pass the first sample emission to the first detector.
3. The apparatus of Claim 2, wherein the first filter comprises an optical filter.
4. The apparatus of Claim 2, wherein the first filter comprises an optical filter having a passband of at least 420 nm to 460 nm.
5. The apparatus of Claim 2, wherein the first filter comprises an optical filter having a passband of at least 430 nm to 490 nm.
6. The apparatus of Claim 2, wherein the first filter comprises an optical filter having a pass band substantially centered at 457 nm and having a bandwidth of at least 10 nm.
7. The apparatus of Claim 2, wherein the first filter comprises an optical filter having a pass band substantially centered at 457 nm and having a bandwidth of at least 20 nm.
8. The apparatus of Claim 2, further comprising a second filter interposed between the sample receptacle and the second detector and configured to substantially pass the second sample emission to the second detector.
9. The apparatus of Claim 8, wherein the second filter comprises an optical filter.
10. The apparatus of Claim 8, wherein the second filter comprises an optical filter having a passband of at least 480 nm to 520 nm.

11. The apparatus of Claim 8, wherein the second filter comprises an optical filter having a passband of at least 500 nm to 580 nm.

12. The apparatus of Claim 8, wherein the second filter comprises an optical filter having a pass band substantially centered at 550 nm and having a bandwidth of at least 10 nm.

13. The apparatus of Claim 8, wherein the second filter comprises an optical filter having a pass band substantially centered at 550 nm and having a bandwidth of at least 20 nm.

14. The apparatus of Claim 1, further comprising an excitation filter interposed between the excitation source and the sample receptacle and configured to substantially pass the excitation emission.

15. The apparatus of Claim 14, wherein the excitation filter comprises an optical filter.

16. The apparatus of Claim 14, wherein the excitation filter comprises an optical filter having a passband of at least 350 nm to 400 nm.

17. The apparatus of Claim 1, wherein the excitation source comprises a broadband light source.

18. The apparatus of Claim 1, wherein the excitation source comprises a xenon light source.

19. The apparatus of Claim 1, wherein the excitation source comprises a narrowband light source.

20. The apparatus of Claim 1, wherein the excitation source comprises a Light Emitting Diode (LED).

21. The apparatus of Claim 1, further comprising a first lens configured to collect the first sample emission onto the first detector.

22. The apparatus of Claim 21, further comprising a second lens configured to collect the second sample emission onto the second detector.

23. The apparatus of Claim 1, further comprising:

a first Analog to Digital Converter (ADC) configured to digitize an output from the first detector;

a second ADC configured to digitize an output from the second detector; and  
wherein the processor calculates the ratio of the detected first sample emission to the detected second sample emission based, at least in part, on outputs from the first and second ADCs.

24. The apparatus of Claim 1, further comprising a memory and wherein the processor stores the ratio of the detected first sample emission to the detected second sample emission in the memory.

25. The apparatus of Claim 1, further comprising an output device and wherein the processor communicates the ratio of the detected first sample emission to the detected second sample emission to the output device.

26. The apparatus of Claim 25, wherein the output device comprises a display.

27. The apparatus of Claim 25, wherein the output device comprises a printer.

28. The apparatus of Claim 25, wherein the output device comprises a computer.

29. The apparatus of Claim 1, wherein the processor is further configured to determine a concentration of a molecule in a sample based, at least in part, on the ratio of the detected first sample emission to the detected second sample emission.

30. The apparatus of Claim 29, wherein the concentration comprises a concentration of unbound free fatty acids.

31. The apparatus of Claim 1, wherein the first detector comprises an optical detector.

32. The apparatus of Claim 1, wherein the first detector comprises a photomultiplier tube.

33. The apparatus of Claim 1, wherein the first detector comprises a photodiode.

34. The apparatus of Claim 1, wherein the first detector comprises a Charge Coupled Device (CCD).

35. The apparatus of Claim 1, wherein the first detector is positioned on an axis different from an axis of the excitation emission.

36. The apparatus of Claim 1, wherein an axis of the first sample emission is the same as an axis of the excitation emission.

37. The apparatus of Claim 1, wherein the first sample emission comprises a fluorescence from a molecule in a bound state and the second sample emission comprises a fluorescence from the molecule in a free state.

38. The apparatus of Claim 1, wherein the first sample emission comprises a fluorescence from a first molecule whose fluorescence changes upon binding with a desired molecule and the second sample emission comprises a fluorescence from a second molecule whose fluorescence does not significantly change in the presence of the desired molecule.

39. A ratio fluorometer for determining a concentration of a target molecule in a sample, the fluorometer comprising:

- a sample receptacle configured to support the sample;
- an excitation light source directed towards the sample receptacle;
- a first optical filter configured to filter a first fluorescence from the sample;
- a first detector configured to detect an output of the first optical filter;
- a first Analog to Digital Converter (ADC) configured to generate a digital representation of an output of the first detector;
- a second optical filter configured to filter a second fluorescence from the sample;
- a second detector configured to detect an output from the second optical filter;
- a second ADC configured to generate a digital representation of an output of the second detector; and
- a processor configured to calculate a ratio, based in part, on the digital representations of the output of the first detector and the output of the second detector and to determine a concentration of the target molecule based, at least in part, on the ratio.

40. The fluorometer of Claim 39, wherein:

- the excitation light source comprises a light source having emissions with wavelengths of 350 nm – 400 nm;
- the first filter comprises a passband of 420 nm – 460 nm; and
- the second filter comprises a passband of 480 nm -520 nm.

41. The fluorometer of Claim 39, wherein:

the excitation light source comprises a light source having emissions with wavelengths of 350 nm – 400 nm;

the first filter comprises a passband of 430 nm – 490 nm; and

the second filter comprises a passband of 500 nm -580 nm.

42. The fluorometer of Claim 39, wherein:

the first filter comprises a passband configured to pass a fluorescence of ADIFAB2 when ADIFAB2 is bound to the target molecule; and

the second filter comprises a passband configured to pass a fluorescence of ADIFAB2 when ADIFAB2 is not bound to the target molecule.

43. The fluorometer of Claim 39, wherein:

the first filter comprises a passband configured to pass a fluorescence of ADIFAB when ADIFAB is bound to the target molecule; and

the second filter comprises a passband configured to pass a fluorescence of ADIFAB when ADIFAB is not bound to the target molecule.

44. A ratio fluorometer for determining a concentration of unbound free fatty acids (FFAu) in a sample, the fluorometer comprising:

a sample receptacle configured to support the sample;

an excitation source;

a first detector configured to detect a first fluorescence of a molecule when the molecule is bound to a ligand of the free fatty acid in the sample;

a second detector configured to detect a second fluorescence of the molecule when the molecule is unbound to the ligand of the free fatty acid in the sample; and

a processor configured to calculate a ratio, based at least in part, on an output of the first detector and an output of the second detector, and based at least in part on the ratio, to determine the FFAu concentration in the sample.

45. The fluorometer of Claim 44, wherein the molecule comprises ADIFAB.

46. The fluorometer of Claim 44, wherein the molecule comprises ADIFAB2.

47. A method comprising:

detecting a first emission from a sample to produce a first detected emission;

detecting a second emission from the sample to produce a second detected emission; and

determining a concentration of a molecule in the sample based at least in part on the ratio of the detected emissions.

48. The method of Claim 47, further comprising exciting the sample with an excitation emission.

49. The method of Claim 48, wherein exciting the sample comprises directing a light source having emissions in a 350 nm – 400 nm wavelength band towards the sample.

50. The method of Claim 47, wherein detecting the first emission from the sample comprises:

filtering an emission from the sample using a first optical filter;

detecting an output of the first optical filter to produce a first detector output;

and

converting the first detector output to a first digital representation.

51. The method of Claim 50, wherein filtering the emission from the sample comprises optically filtering the emission with a filter passband including 420 nm - 460 nm.

52. The method of Claim 50, wherein filtering the emission from the sample comprises optically filtering the emission with a filter passband including 430 nm - 490 nm.

53. The method of Claim 50, wherein filtering the emission from the sample comprises optically filtering the emission with a filter passband centered approximately at 457 nm.

54. The method of Claim 50, wherein detecting the second emission from the sample comprises:

filtering the emission from the sample using a second optical filter;

detecting an output of the second optical filter to produce a second detector output; and

converting the second detector output to a second digital representation.

55. The method of Claim 54, wherein filtering the emission from the sample comprises optically filtering the emission with a filter passband including 480 nm - 520 nm.

56. The method of Claim 50, wherein filtering the emission from the sample comprises optically filtering the emission with a filter passband including 500 nm - 580 nm.

57. The method of Claim 50, wherein filtering the emission from the sample comprises optically filtering the emission with a filter passband centered approximately at 550 nm.

58. A method of determining a concentration of a target molecule in a sample, the method comprising:

- exciting the sample with an excitation light source;
- detecting, using a first detector, a first fluorescence from the sample;
- converting an output of the first detector to a first digital representation;
- detecting, using a second detector, a second fluorescence from the sample;
- converting an output of the second detector to a second digital representation;
- calculating a ratio based, at least in part, on the first digital representation and the second digital representation; and
- determining the concentration of the target molecule based, at least in part, on the ratio.

59. The method of Claim 58, wherein exciting the sample comprises directing a light source having an emission bandwidth of at least 350 nm – 400 nm at the sample.

60. The method of Claim 58, wherein:

- detecting the first fluorescence from the sample comprises detecting an emission from the sample in a 420 nm – 460 nm wavelength band; and
- detecting the second fluorescence from the sample comprises detecting an emission from the sample in a 480 nm – 520 nm wavelength band.

61. The method of Claim 58, wherein:

- detecting the first fluorescence from the sample comprises detecting an emission from the sample in a 430 nm – 490 nm wavelength band; and
- detecting the second fluorescence from the sample comprises detecting an emission from the sample in a 500 nm – 580 nm wavelength band.

62. The method of Claim 58, wherein:

detecting the first fluorescence from the sample comprises detecting a first emission from molecules bound to ligands; and

detecting the second fluorescence from the sample comprises detecting a second emission from the sample molecules not bound to ligands.

63. The method of Claim 58, wherein:

detecting the first fluorescence from the sample comprises detecting a first emission from ADIFAB2 molecules bound to ligands in the sample; and

detecting the second fluorescence from the sample comprises detecting a second emission from ADIFAB2 molecules not bound to ligands in the sample.

64. A method of determining a concentration of unbound free fatty acids (FFAu) in a sample, the method comprising:

determining a fluorescence of bound molecules from the sample using a first detector;

determining a fluorescence of unbound molecules from the sample using a second detector; and

determining the concentration of FFAu in the sample based, at least in part, on a ratio of the fluorescence of bound molecules to the fluorescence of unbound molecules.

65. A measurement apparatus comprising:

a means for receiving a sample;

a means for exciting the sample;

a first means for detecting first sample emissions;

a first means for generating a first digital output corresponding to an output from the first means for detecting;

a second means for detecting second sample emissions;

a second means for generating a second digital output corresponding to an output from the second means for detecting; and

a processor configured to determine a concentration of target molecules in the sample based, at least in part, on a ratio of the first digital output to the second digital output.



66. A measurement apparatus, comprising:
- a first means for filtering a sample emission to produce a first filtered emission;
  - a first means for detecting the first filtered emission and producing a first detected emission value;
  - a second means for filtering the sample emission to produce a second filtered emission;
  - a second means for detecting the second filtered emission and producing a second detected emission value; and
  - a processor coupled to the first means for detecting and the second means for detecting and configured to determine, based in part on a ratio of the first detected emission value to the second detected emission value, a concentration value.
67. The measurement apparatus of Claim 66, wherein the first means for detecting and the second means for detecting comprise a single broadband detector.
68. The measurement apparatus of Claim 67, wherein the first means for filtering and second means for filtering are successively placed in front of the single broadband detector.
69. The measurement apparatus of Claim 66, wherein the processor in combination with the first means for detecting and second means for detecting determines the ratio of the first detected emission value to the second detected emission value with a coefficient of variation of less than 1.0%.
70. The measurement apparatus of Claim 66, further comprising a temperature sensor configured to produce a temperature value, and wherein the processor determines the concentration value, based in part, on the temperature value.
71. A method of determining a concentration of a target molecule in a sample, the method comprising:
- detecting a first fluorescence from the sample in a first bandwidth to produce a first detected value;
  - detecting a second fluorescence from the sample in a second bandwidth to produce a second detected value;

calculating a ratio based, at least in part, on the first detected value and the second detected value; and

determining the concentration of the target molecule based, at least in part, on the ratio.

72. The method of Claim 71, wherein the first bandwidth comprises a first interference absorbance bandwidth having a first optical density and the second bandwidth comprises a second interference absorbance bandwidth having a second optical density substantially equal to the first optical density.

73. The method of Claim 71, wherein an optical density of an interference absorbance spectrum is substantially equal in the first and second bandwidths.

74. The method of Claim 73, wherein the interference absorbance spectrum comprises a hemoglobin absorbance spectrum.

75. The method of Claim 71, further comprising verifying an accuracy of the concentration of the target molecule.

76. The method of Claim 75, wherein verifying the accuracy of the concentration of the target molecule comprises:

preparing a control solution having a known concentration;

determining a concentration of the control solution; and

comparing the concentration of the control solution to the known concentration.

77. The method of Claim 75, wherein verifying the accuracy of the concentration of the target molecule comprises:

determining a concentration of a standard; and

comparing the concentration of the standard to a predetermined concentration range.

78. The method of Claim 77, wherein the standard comprises a solid standard including a solid fluorophore.

79. The method of Claim 77, wherein the standard comprises a solution standard including a fluorophore in a defined solution.

80. A method of calibrating a ratio fluorometer; the method comprising:

determining in a calibrating ratio fluorometer a ratio of fluorescence of a sample;

determining in a second ratio fluorometer an uncorrected ratio of fluorescence of the sample; and

adjusting a detector output such that the uncorrected ratio of fluorescence is substantially equal to the ratio of fluorescence.

81. The method of Claim 80, wherein determining the ratio of fluorescence of the sample comprises determining a ratio of a fluorescent molecule in the presence of substantially no target molecules.

82. The method of Claim 80, wherein determining the ratio of fluorescence of the sample comprises determining a ratio of an ADIFAB sample that is substantially devoid of free fatty acids.